



**NTP**  
National Toxicology Program

## **Draft NTP Technical Report GMM15 on Senna**

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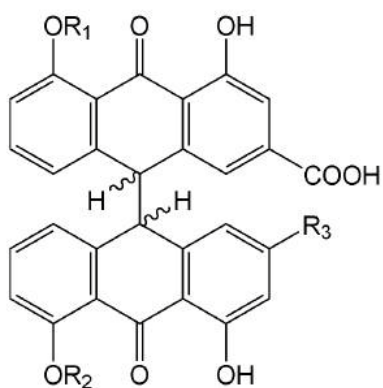
## Senna

- Leaf and pod from *Senna alexandria* P. Mill
- Laxative (over-the-counter drug category III)
- Flavoring Agent
- Nomination by the Food and Drug Administration (FDA)
  - Wide use
  - Positive genotoxicity *in vitro* for components/metabolites
  - Unknown carcinogenic potential
  - p53 (+/-) mouse model

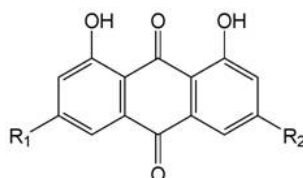




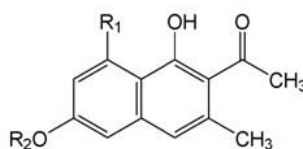
## Components of Senna



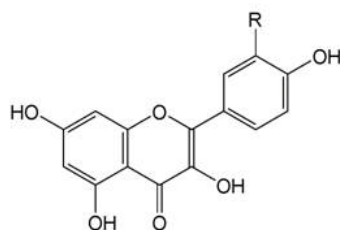
Dianthrone  
sennosides  
sennidins



Anthraquinones  
Aloe-emodin  
Rhein



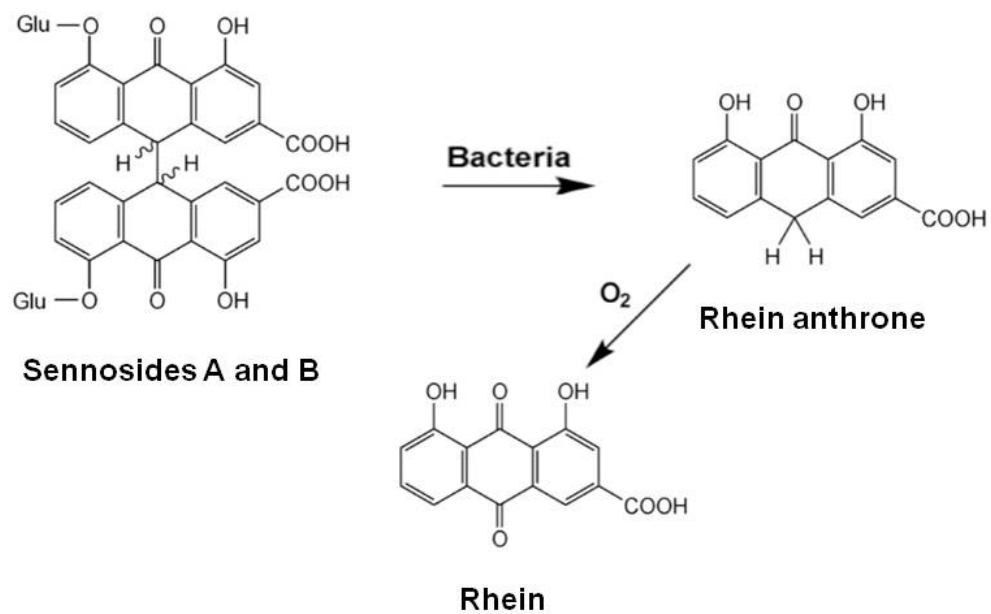
Naphthalenes  
Torachrysone



Flavonoids  
Kaempferol (glycosides)  
Quercetin



## Metabolism of Sennosides A and B





## Study Design

- Genetic toxicity studies (*in vitro* & *in vivo*)
- Range finding study – C57BL/6NTac mice
- 40 wk Study – C3B6.129F1-*Trp53*<sup>tm1Brd</sup> (+/-) mice
  - C3H/HeNTac (♀) X C57BL/6.129Sv-*Trp53*<sup>tm1Brd</sup> N12 (♂)
  - Low spontaneous tumor incidence up to 9 months of age
  - Decreased tumor latency compared to wild type mice
  - Accepted by the FDA for testing potential genotoxicants for carcinogenicity



## Genotoxicity Test Result

- Senna
  - *In vitro* (bacteria mutagenicity assays)
    - Weak and inconsistent results
    - Metabolic activation generally required for positive responses
  - *In vivo* (male mice)
    - No increase in micronucleated erythrocytes
- Sennosides A and B – *in vitro*
  - Not mutagenic in bacteria
- Rhein – *in vitro*
  - Mutagenic in bacteria in the presence of metabolic activation



## **5-Week Study**

- Male and female C57BL/6NTac mice
- 5 animals/sex/group
- Feed (0, 625, 1250, 2500, 5000, 10,000 ppm) for 29 days
  
- All mice survived
- No effect on body weight

## 5-Week Study – C57BL/6NTac Mice

Dose (ppm)	0	625	1,250	2,500	5,000	10,000
<b>Male</b>						
<b>Cecum</b>						
epithelial hyperplasia	0	2 (1.0)	1 (1.0)	1 (1.0)	3 (1.0)	5** (1.8)
<b>Colon</b>						
epithelial hyperplasia	0	0	1 (1.0)	1 (1.0)	4* (1.3)	5** (2.0)
<b>Female</b>						
<b>Cecum</b>						
epithelial hyperplasia	0	0	0	2 (1.0)	4* (1.0)	5** (1.8)
<b>Colon</b>						
epithelial hyperplasia	0	0	0	1 (1.0)	5** (1.4)	5** (3.0)
<b>Rectum</b>						
epithelial hyperplasia	0	0	0	0	0	3 (1.0)

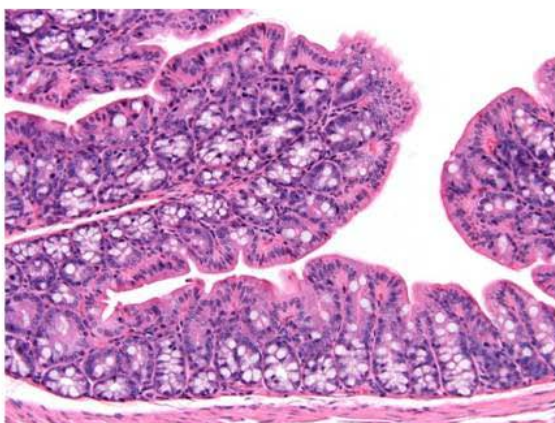
N=5; \*P≤0.05; \*\*P≤0.01





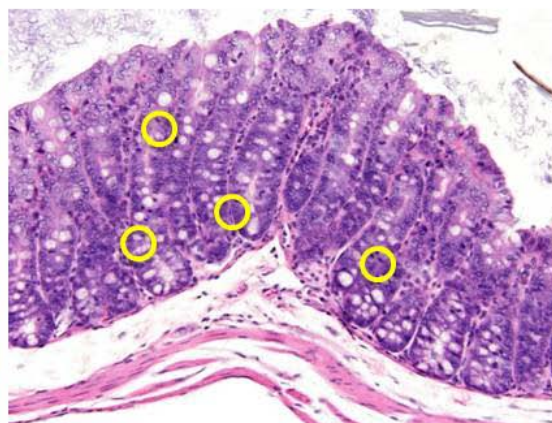
## 5-Week Study – C57BL/6NTac Mice (Colon)

**Control (♀)**



**10,000 ppm (♀)**

Epithelial hyperplasia



 Mitotic figures



## 40-Week Study

- Male and female C3B6.129F1-*Trp53*<sup>tm1Brd</sup> (+/-) mice
- 25 animals/sex/group
- Feed (0, 100, 300, 1000, 3000, 10,000 ppm) for 40 weeks

Dose (ppm)	0	100	300	1,000	3,000	10,000
<b>Survival</b>						
Male	25	24	25	25	23	24
Female	23	23	23	22	22	24
<b>Body weight (% control)</b>						
Male	-	101	104	98	101	95
Female	-	99	96	99	97	93

N = 25

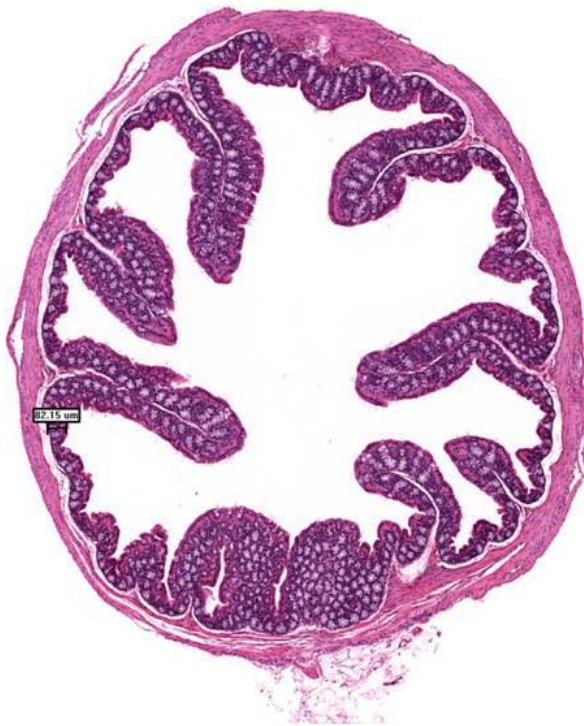
## 40-Week Study – C3B6.129F1-*Trp53*<sup>tm1Brd</sup> (+/-) Mice

Dose (ppm)	0	100	300	1,000	3,000	10,000
<b>Male</b>						
Cecum epithelial hyperplasia	0/25	0/25	0/25	0/25	0/23	22/25** (1.4)
Colon epithelial hyperplasia	0/25	0/25	0/25	0/25	3/24 (1.3)	25/25** (2.8)
Rectum epithelial hyperplasia	0/24	0/25	0/25	0/25	0/24	1/25 (2.0)
<b>Female</b>						
Cecum epithelial hyperplasia	0/25	0/25	0/25	0/25	0/25	19/25** (1.3)
Colon epithelial hyperplasia	0/25	0/25	0/25	0/25	7/25** (1.0)	25/25** (2.7)
Rectum epithelial hyperplasia	0/25	0/25	0/25	0/25	0/25	1/25 (1.0)

\*\* P≤0.01

**40-Week Study – C3B6.129F1-*Trp53*<sup>tm1Brd</sup> (+/-) Mice (Colon)**

**Control (♂)**



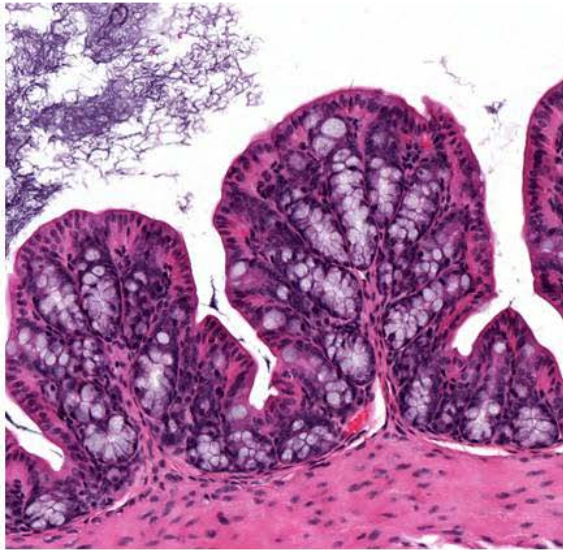
**10,000 ppm (♂) – Epithelial Hyperplasia**



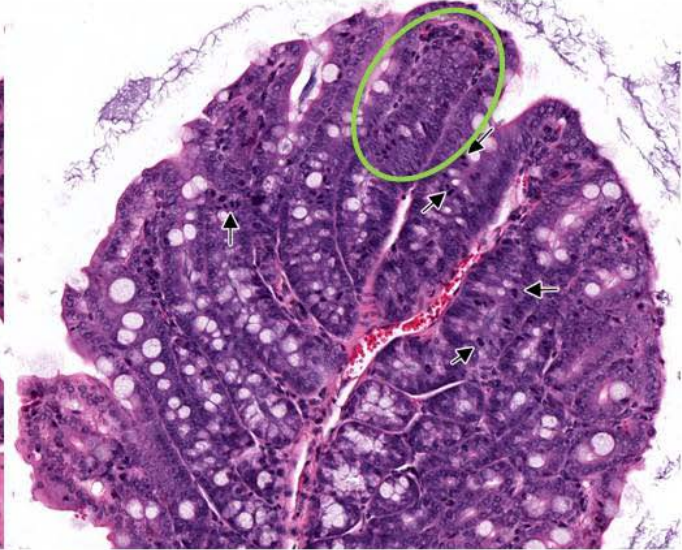




40-Week Study – C3B6.129F1-*Trp53*<sup>tm1Brd</sup> (+/-) Mice (Colon)

Control (♂)



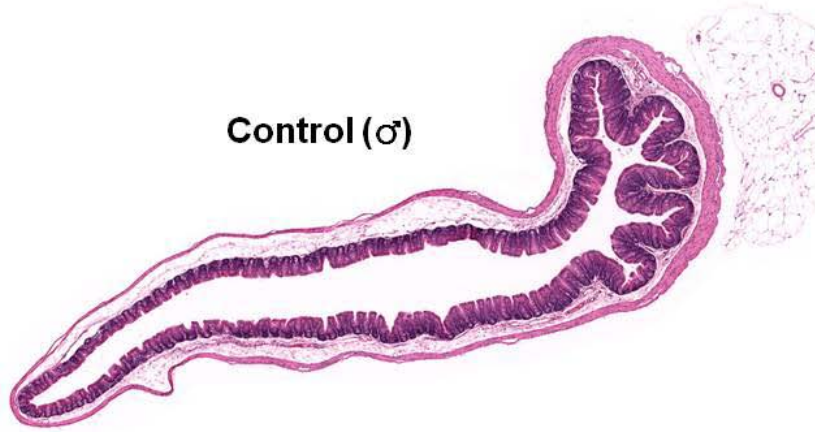
10,000 ppm (♂) – Epithelial Hyperplasia



-  Crowded with cell nuclei
-  Mitotic figures

**40-Week Study – C3B6.129F1-*Trp53*<sup>tm1Brd</sup> (+/-) Mice (Cecum)**

**Control (♂)**



**10,000 ppm (♂) – Epithelial Hyperplasia (grade 3)**





## Conclusion

- Under the conditions of this 40-week feed study, there was ***No evidence of carcinogenic activity of senna in male or female C3B6.129F1-Trp53<sup>tm1Brd</sup> N12 haploinsufficient mice*** exposed to 100, 300, 1,000, 3,000, or 10,000 ppm
- Senna induced epithelial hyperplasia of the large intestine (colon and cecum) in male and female mice

## 40-Week Study – C3B6.129F1-*Trp53*<sup>tm1Brd</sup> (+/-) Mice

Dose (ppm)	0	100	300	1,000	3,000	10,000
<b>Male</b>						
<b>Liver</b>						
hepatocellular adenoma <sup>a</sup>	1	0	5	0	2	1
<b>Bone</b>						
osteosarcoma	0	0	0	3	2	0
osteoma or osteosarcoma <sup>b</sup>	0	0	1	3	2	0
<b>Female</b>						
<b>Bone</b>						
osteosarcoma <sup>c</sup>	2	2	1	3	0	2

N = 25

<sup>a</sup> Historical control: 5/76 (6.5%; 4-11.5%)

<sup>b</sup> Historical control: 5/77 (6.5%; 0-12%)

<sup>c</sup> Historical control: 3/76 (3.9%; 0-8%)